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Article

Neo- and Paleo-Limnological Studies on Diatom and Cladoceran Communities of Subsidence Ponds Affected by Mine Waters (S. Poland)

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Abstract: Plankton assemblages can be altered to different degrees by mining. Here, we test how diatoms and cladocerans in ponds along a river in southern Poland respond to the cessation of the long-term Pb-Zn mining. There are two groups of subsidence ponds in the river valley. One of them (DOWN) was contaminated over a period of mining, which ceased in 2009, whereas the other (UP) appeared after the mining had stopped. We used diatoms and cladocerans (complete organisms in plankton and their remains in sediments) to reveal the influence of environmental change on the structure and density of organisms. The water of UP pond was more contaminated by major ions (SO_4^{2-}, Cl^-) and nutrients (NO_3^-, PO_4^{3-}) than the DOWN ponds. Inversely, concentrations of Zn, Cd, Cu and Pb were significantly higher in sediment cores of DOWN ponds in comparison to those in the UP pond. Ponds during mining had higher diversity of diatoms and cladocerans than the pond formed after the mining had stopped. CCA showed that diatom and cladoceran communities related most significantly to concentrations of Pb in sediment cores. Comparison of diatom and cladoceran communities in plankton and sediment suggests significant recovery of assemblages in recent years and reduction of the harmful effect of mine-originating heavy metals. Some features of ponds such as the rate of water exchange by river flow and the presence of water plants influenced plankton communities more than the content of dissolved heavy metals.

Keywords: Zn-Pb mine; subsidence ponds; physico-chemical water variables; subfossil; Cladocera; diatoms; heavy metals; CCA analyses; anthropogenic impact

1. Introduction

Mining industry influences the aquatic environment. Among environmental effects, draining of mines and mine tailings as well as leaching of spoil heaps have been recognized as particularly harmful for aquatic organisms. The impact of such pollution can be the most distinct in small catchments, receiving large amounts of mine drainage where dilution with natural waters is limited. Mine waters usually contain many compounds in potentially harmful amounts, inducing synergistic effects on organisms [1–4]. For example, heavy metals (Cu, Pb, Zn) could be less toxic for biota in water (e.g., Cladocera) at high content of cations like Ca^{2+} and Mg^{2+} [5,6]. It was also found that in systems with elevated metal concentrations and acidic pH, species richness decreases and the number of taxa is low [4,7–9].

Chemical quality of water bodies (e.g., lakes, rivers, dam reservoirs) receiving mining waters has been monitored in many aquatic systems, but their long term ecological impacts are only rarely estimated. Most data show a negative effect of pollution (especially by heavy metals) on planktonic organisms [4,10]. However, some observations show that algae and zooplankton can adapt to prolonged heavy metal contamination e.g., [11–14]. For example, in small fishponds in a partially reclaimed area impacted by the lead–zinc mine Matylda (southern Poland, Chrzanów area), the influence of heavy metals remains a minor factor, although small amounts of teratogenic forms of pyto- and zooplankton have been found [12,13].

Sediments from lakes are environmental libraries, abundant in information about the history of catchments and their ecosystems. Paleolimnological studies of these sediments polluted by mining, can help to reconstruct changes of environmental conditions. As a rule, sedimentary geochemistry and associated subfossil remains of biological communities (e.g., cladoceran crustacea, diatom algae) are used to assess the natural pre-disturbance variability, the impact of the disturbance and post-disturbance dynamics [4,15]. Based on ecological preferences of particular organisms they can be used to assess the impacts of pollution on biological communities [4,16]. Because of different habitat preferences, biological remains of taxa can be sources of information about differences between depositional subenvironments and their changes over time (e.g., [4,17]).

Diatoms and cladocerans are most often used as indicators in paleoenvironmental reconstructions because of good preservation of chitinous and siliceous cell walls and well-established environmental preferences of a number of taxa [4,18]. Diatoms are a base element of trophic food chain with observed biomagnification of heavy metals [19]. They are good bioindicators of metal toxicity in fluvial and lentic systems [15,19–22]. Diatoms and cladocerans have great potential in paleolimnological pollution studies because of their sensitivity to changes in water quality and their location at the basis of food-webs. Heavily impacted aquatic environments can be dominated by metal-resistant diatoms and cladocerans species or with species of broad ecological tolerance [4].

The aim of our study is to recognize changes in the species composition of diatoms and cladocerans in response to Zn-Pb mining cessation, recorded in water and bottom sediments of subsidence ponds situated on the Chechło River floodplain (southern Poland). We compared these communities in subsidence ponds active during the period of mining and in a subsidence pond inundated after the mining cessation assuming that the younger one will be less polluted with heavy metals. The first hypothesis assumes that diatom and cladoceran communities are not affected by concentration of heavy metals in water of subsidence ponds. The second hypothesis assumes that regeneration of the diatom and cladoceran communities are influenced by high heavy metal concentrations in the sediments of ponds in the river valley downstream of the mine waters discharge. The present study may be a key to understanding factors controlling ecosystem recovery from long-term disturbance. We address this by comparison of diatoms and cladocerans species living in water with their past communities using remains preserved in sediments of subsidence ponds and by correlation of their composition with present water physico-chemical variables and records of metal contamination in sediments.

2. Materials and Methods

2.1. Study Area

The ponds are situated in the middle course of the Chechło River. This area was impacted by the discharge of mine waters from a Zn-Pb mine (Trzebionka) and by the other industrial and municipal sewage from the two towns, Trzebinia and Chrzanów [23,24]. Over the investigated period, Zn-Pb mine was the dominating source of heavy metals in this river system whereas pollution from the two towns continued despite some variability [23]. We distinguished two research areas about 1 km apart: a large subsidence pond that emerged after the closure of the mine (UP) and several subsidence depressions ponded during the peak of the ore exploitation (DOWN) (Figure 1). Their areas range from 0.5 to ca. 5 ha and the average depth ranges between 1 and 2 m. Some (ca. 20–50%) of the ponds are overgrown with macrophytes.

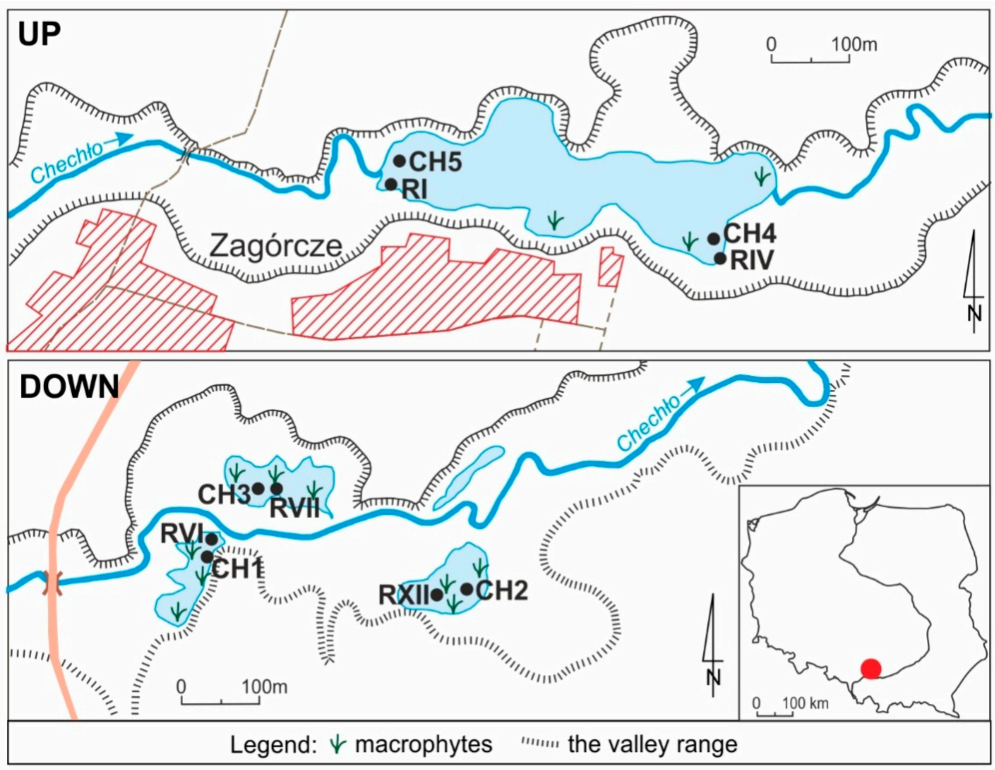

Figure 1. Sampling area-UP: Subsidence pond formed after the mine closure: water and plankton samples—CH4, CH5, and sediment cores: RI, RIV; DOWN: Subsidence ponds formed during peak exploitation: water and plankton samples–CH1, CH2, CH3 and sediment cores: RVI, RVII, RXII.

2.2. Sampling and Measurements

Samples for water, diatom, and cladoceran analyses were taken from sites CH1, CH2, CH3 (DOWN ponds), CH4 and CH5 (UP pond) four times a year (April, July, September and October 2016) (Figure 1). Core samples (UP pond: RI, RIV; DOWN pond: RVI, RVII, RXII) for heavy metals concentration, diatoms, and cladocerans were taken once in 2016, close to the same sites where water and plankton samples were collected (Figure 1). Cores were sampled using a multisampler piston corer with diameter 4.5 cm (Eijkelkamp, Giesbeek, Netherlands). The methodology for developing samples of collected cores is described in Pociecha et al. [25].

2.2.1. Physico-Chemical Water and Sediment Core Analyses

In the water samples pH, conductivity, anions Cl^-, NO_3^-, PO_4^{3-}, cations NH_4^+, Mg^{2+}, Ca^{2+}, and heavy metals Cd, Cu, Pb, and Zn concentrations were analyzed. pH and conductivity were measured in situ using a WTW (Multi 340i/SET 2, Wissenschafttlich-Technische Werkstacten 823362 Weiheim, Germany) handheld multimeters. For anion and cation analysis water samples were filtered through a 0.45-μm pore-sized syringe filter. They were analyzed within 48 h from sampling by ion chromatography (DIONEX, IC25 and ICS–1000, Dionex Corporation, Sunnyvale, CA, USA). Concentrations of Cd, Pb, Cu, and Zn in total and dissolved phases (after filtration through 0.45-μm filter) were measured by atomic absorption spectroscopy (ASA), using a Varian Spectra AA–20 with a Graphite Furnace (Varian 20, Varian Techtron PTY Limited, Mulgrave, Victoria, Australia). Standard reference materials for water SPS–SW1 Batch 12, National Institute of Standards and Technology (USA), was used to determine the accuracy of metal analyses in the water samples. Water hardness was calculated as a sum of Ca and Mg ions.

Sediment samples for heavy metal analysis were dried at 105 °C and sieved through a 0.063 mm sieve. Then they (0.5 g) were digested with 10 cm^3 of 65% HNO_3 and 2 cm^3 of 30% H_2O_2 (both analytical grade) using a microwave digestion technique [14]. The Cd, Pb, Zn, and Cu concentrations were measured with an inductively coupled plasma-mass spectrometer (Perkin Elmer ELAN 6100) in the certified Hydrogeochemical Laboratory (AGH University, Krakow, Poland) according to the standard certified analytical quality control procedure (PN-EN ISO 17294-1:2007).

2.2.2. Diatoms in Water and Sediment

In the field, 10 L of water was collected with a 10-µm plankton net. The core samples for diatoms analysis were taken close to the same sites where the plankton was collected. The 1 cm^3 samples were taken at 10 cm intervals immediately after retrieval. The samples for diatom analysis were boiled in concentrated H_2O_2, treated with 10% HCl and washed several times with distilled water in order to remove organic matter. The cleaned diatom material was air dried on cover slips and mounted in Naphrax Mountant, Brunel Microscopes Ltd. Observations of the diatoms were performed with a Nikon Eclipse 80i microscope equipped with oil immersion and differential interference contrast. The identification of diatoms was based mainly on Krammer and Lange-Bertalot [26–29], and specific taxonomic publications. Taxonomic identifications were made to the lowest possible level. Diatoms collected from the plankton and sediment cores were processed following a procedure—a minimum of 400 valves were counted from every subsample. Only taxa that exceeded 0.2% of the relative abundance were used for statistical analysis. Diatom data were expressed as relative abundance reflecting changes in the assemblage structure, indicating potential fluctuations in the environment. In order to reconstruct the environmental conditions in plankton and during the deposition of the sediments studied, diatoms were grouped according to their environmental requirements. Here we used a term—sedimentary diatoms—for all taxa found in the cores.

2.2.3. Cladocera in Water and Sediment

Samples for living Cladocera were taken from the central point of each pond. For taxonomic identification and quantitative analyses, samples were collected using a 5-L Ruttner sampler. In the field, 10 L of water samples (2 replicate, 5 L samples) were concentrated with a 50-µm plankton net. For identification and counting of zooplankton species, five replicate sub-samples were analyzed microscopically (×100 or ×200) in the chamber volume of 0.5 mL^{-1}. Taxonomic analyses of Cladocera were conducted using the identification keys [30,31]. The density of individuals were calculated per liter. Subfossil sediment Cladocera were prepared according to Frey [32]. One centimeter cube of fresh homogenized sediment was taken from the particular depths from each core for cladoceran analysis. Laboratory methods were described in a previous publication [25]. Taxa were identified and counted at 200× or 400× magnification under a Nikon 50i microscope. All skeletal parts were counted: headshields, shells, postabdomens, postabdominal claws, ephippia, and filtering combs. The most abundant body part for each taxon was chosen to represent the number of individuals. The results of qualitative and quantitative analyses are presented in diagrams, in which an absolute number of specimens was calculated for 1 cm^3 sediment volume. Identification of the species was based on Frey [33] and Szeroczyńska and Sarmaja-Korjonen [34].

2.3. Statistical Analyses

In order to find the significant differences in the values of studied physicochemical variables in water between UP (CH4-CH5) and DOWN (CH1-CH3) ponds Mann–Whitney test was used. Differences in metal concentrations in the sediments between separate groups (as defined by hierarchical cluster analysis) were evaluated by Mann-Whitney test. To determine the degree of sediment contamination by heavy metals the index of geoaccumulation (I_{geo}) was calculated according to Müller [35] equation: $I_{geo} = \log_2(Cn/1.5Bn)$, where: Cn is the mean concentration of an element in the bottom sediment, and Bn is the geochemical background of the element in the shale [36].

According to values of the IgeoMüller [35] we distinguished seven classes of sediment contamination; Class 0, $I_{geo} \leq 0$, uncontaminated; Class 1, $0 < I_{geo} \leq 1$, uncontaminated to moderately contaminated; Class 2, $1 < I_{geo} \leq 2$, moderately contaminated; Class 3, $2 < I_{geo} \leq 3$, moderately to heavily contaminated; Class 4, $3 < I_{geo} \leq 4$, heavily contaminated; Class 5, $4 < I_{geo} \leq 5$, heavily to extremely contaminated; Class 6, $I_{geo} > 5$, extremely contaminated. Shannon's diversity index was applied in order to evaluate the diversity of Cladocera and Bacillariophyta (diatoms) (MultiVariate Statistical Package (MVSP) 3.1 program, Kovach Computing Services).

We used Spearman's correlation coefficient to investigate the relationship between cladocerans and diatoms occurrence and the content of heavy metals in the sediments and the physico-chemical characteristics of the waters (Statistica 13 program).

Cladocera and diatom communities were classified based on their similarities using the hierarchical clustering method (UPGMA). The clustering classification was obtained using the MVSP 3.1 program.

The significance of the differences between ponds created during mine exploitation (DOWN) and those created after the mine was closed (UP) and the density of diatoms and cladocerans were evaluated using Mann–Whitney U test (Statistica version 13.1, Dell version).

Canonical correspondence analysis (CCA) was used to analyze species and environmental data. We performed DCA analysis (detrended correspondence analysis) based on the length of the gradient expressed in standard deviation (SD) units. For DCA and CCA analysis, the data was log-transformed ln (x + 1) and centered. In the CCA analysis a forward selection was used to reduce the set of environmental variables. Analysis was performed on cladocerans and diatoms data and sediments samples to identify the changes in the water bodies and to show the relationships between the environmental variables and the distribution of studied organisms. The statistical significance, as well as the statistical significance of canonical axes, was accessed using the Monte Carlo permutation test for 499 repetitions (CANOCO for Windows 4.5 program).

3. Results

3.1. Physico-Chemical Variates in Waters of Subsidence Ponds

The water of ponds was from circumneutral to slightly alkaline pH (6.7–7.9). Conductivity ranged between 412 and 892 $\mu S\ cm^{-1}$, contents of major anions (mg/dm^3) varied: Cl^- 25.1–82.8, SO_4^{2-} 25.2–165.7, HCO_3^- 104–265, cations Mg^{2+} 10.8–26.5, Ca^{2+} 38.3–86.1, total hardness 2.8–6.3 mval/dm^3, alkalinity 1.7–4.3 mval/dm^3, NO_3^- nd (not detected)–21.2 and PO_4^{3-} 0.10–1.52. The result of Mann–Whitney test indicated significantly higher values of conductivity, pH, concentrations of ions Cl^-, SO_4^{2-}, Mg^{2+}, NO_3^-, PO_4^{3-} in the UP pond when compared to those at the DOWN ponds (Tables 1 and 2). Some parameters show differences between the sites at the DOWN ponds. The lowest medians of conductivity, ions SO_4^{2-}, Cl^-, and PO_4^{3-} were found at site CH3 (Table 1). The highest variability of concentrations of the major ions (with the exception of hydrocarbonates), nutrients, and total hardness was found in the UP pond (site CH5) (Table 1).

Total heavy metal concentrations in water varied in following ranges (in µg/dm^3): Cd nd–4.6, Pb 1.0–20.3, Cu nd–5.0, and Zn 20.0–91.3, while metals in dissolved phase varied: Cd nd–0.53, Pb 0.1–7.3, Zn nd–47.1. In the studied waters the concentrations of Cd total was usually < 0.6 µg/dm^3, Pb total < 5.5 µg/dm^3, Zn total < 45 µg/dm^3 (70%, 70%, and 60% of cases, respectively), while Cd dissolved < 0.13 µg/dm^3, Pb dissolved < 2 µg/dm^3, and Zn dissolved < 30 µg/dm^3 (and 65%, 75%, and 80% of cases, respectively). The concentrations of Zn total were significantly higher in water of the UP pond than those in DOWN ponds (Table 2). The highest metal concentrations in water appeared in different seasons and sites. Maximum concentrations of Cd total and dissolved and Pb total and dissolved in all waters were found in August (with the exception of Pb total in the pond CH1 and Pb dissolved in pond CH4). Then, the concentrations of Pb (total and dissolved) were ca. 2–3 times higher in ponds CH2 and CH3 than in CH4 and CH5. The highest concentrations of total Cd and Zn were found at site CH5, Pb at site CH2, while Cu at site CH4 (Table 1).

Table 1. Physico-chemical parameters (median, range) of the waters of the subsidence ponds.

Parameter	Sites									
	CH1		CH2		CH3		CH4		CH5	
	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
pH	6.8	6.7–7.1	6.8	6.7–7.5	7.1	7.0–7.4	7.3	7.0–7.5	7.5	7.1–7.9
Conductivity (µS/cm)	502	464–535	515	434–588	460	412–470	838	825–862	848	773–892
Cl^- (mg/dm^3)	38.2	33.3–42.6	40.5	29.8–46.0	30.2	25.1–32.5	70.1	38.1–76.3	65.3	39.4–82.8
SO_4^{2-} (mg/dm^3)	61.0	53.6–82.4	64.0	53.3–81.6	42.7	25.2–50.3	150.7	80.4–156.1	151.8	70.8–165.7
HCO_3^- (mg/dm^3)	175	167–217	174	147–241	180	176–200	204	104–265	202	112–236
Ca^{2+} (mg/dm^3)	53.3	48.5–64.3	52.6	45.9–70.0	56.2	51.4–61.8	80.8	39.4–86.1	83.5	38.3–85.6
Mg^{2+} (mg/dm^3)	15.0	13.5–17.6	13.1	11.6–17.7	13.8	12.2–14.2	22.5	12.0–24.4	23.6	10.8–26.5
NO_3^- (mg/dm^3)	9.4	5.1–15.6	0.6	0.2–2.2	1.8	0.7–4.3	6.7	3.8–9.4	10.9	6.8–21.2
PO_4^{3-} (mg/dm^3)	0.14	0.10–0.18	0.29	0.12–1.28	0.29	0.06–0.71	0.60	0.27–1.10	0.49	0.22–1.52
Total hardness (mval/dm^3)	3.8	3.6–4.7	3.7	3.3–4.9	3.9	3.7–4.2	5.9	3.0–6.2	6.2	2.8–6.3
Alkalinity (mval/dm^3)	2.9	2.7–3.6	2.9	2.4–4.0	3.0	2.9–3.3	3.3	1.7–4.3	3.3	1.8–3.9
Cd total (µg/dm^3)	0.60	0.15–0.87	1.00	nd–1.80	0.33	nd–0.51	0.38	0.16–1.00	0.56	0.48–4.60
Cd dissolved (µg/dm^3)	0.09	0.07–0.53	0.18	nd–0.31	0.30	nd–0.39	0.06	0.06–0.39	0.22	0.06–0.47
Pb total (µg/dm^3)	2.4	2.1–2.6	4.9	1.2–20.3	4.6	3.0–12.9	1.3	1.0–6.9	5.3	2.7–7.7
Pb dissolved (µg/dm^3)	1.5	0.9–2.4	0.7	0.2–5.0	1.7	1.6–7.3	1.0	0.1–2.3	1.2	0.5–3.3
Cu total (µg/dm^3)	2.5	2.0–3.0	1.5	1.0–5.0	2.00	nd–3.0	2.0	nd–6.0	4.0	nd–4.0
Zn total (µg/dm^3)	36.0	29.7–37.6	28.3	22.1–47.6	35.9	20.0–68.1	47.7	27.2–79.0	75.6	40.8–91.3
Zn dissolved (µg/dm^3)	24.3	17.6–28.4	19.3	nd–23.6	21.6	nd–24.3	23.6	21.5–38.1	39.5	27.0–47.1

Table 2. Significant differences in the values of studied physico-chemical parameters in water between the UP (CH4, CH5) and DOWN (CH1-CH3) ponds (Mann–Whitney test). Only significant differences are given.

Parameter	Z	*p*
pH	2.546	0.010
Conductivity	3.665	0.000
Cl^-	3.047	0.002
SO_4^{2-}	3.356	0.000
Mg^{2+}	1.967	0.049
NO_3^-	2.198	0.027
PO_4^{3-}	2.198	0.027
Zn total	2.507	0.012

3.2. Heavy Metals in Sediments of Subsidence Ponds

Metal concentrations in the sediment cores significantly varied (in µg/g): Cd 6.1–612.0, Pb 302.6–10,223, Cu 21.4–397, and Zn 506.7–23,081 (Figure 2). Metal concentrations in the cores RVI, RVII, and RXII (0–10 cm strata) were from a few to several dozen times higher in the DOWN ponds than those in cores RI and RIV from the UP pond (Figure 2). Metal concentrations in the lower and/or middle strata (10–20 and 20–30 cm) of core RXII were similar to those in the UP pond (Figure 2).

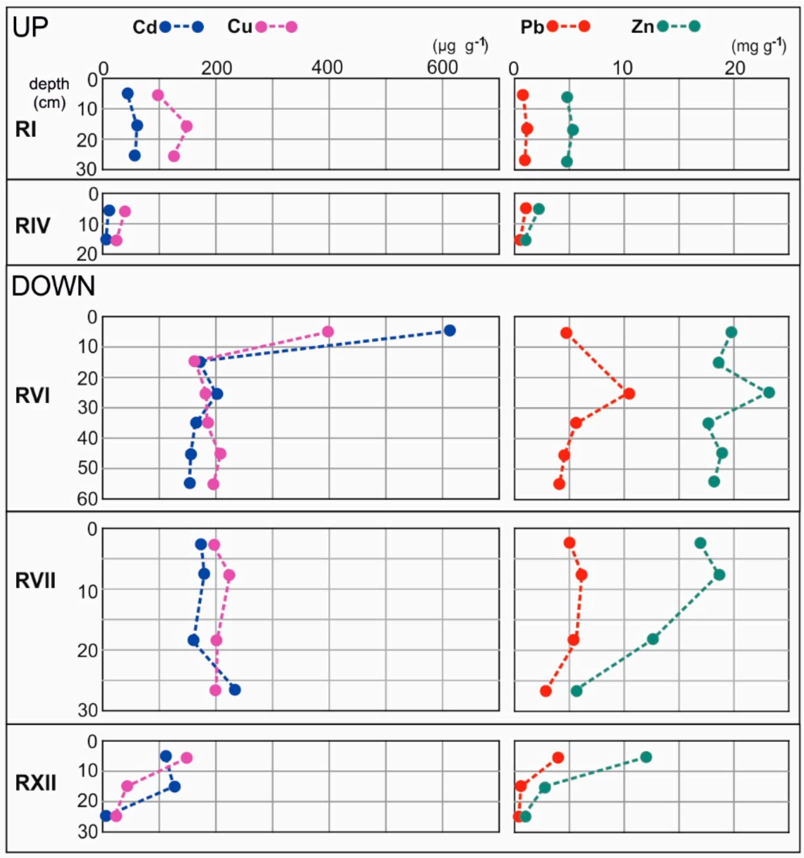

Figure 2. Heavy metal concentrations in the sediment cores in subsidence ponds (UP: Subsidence pond formed after the closure of the mine; cores: RI, RIV; DOWN: Subsidence ponds formed during peak of exploitation; cores: RVI, RVII, RXII).

According to the geoaccumulation index, the 0–10 cm strata of sediment cores RVI, RVII, RXII, and of core RI (with the exception of Pb were extremely contaminated by Zn, Cd, and Pb ($I_{geo} > 5$, class 6) (Table 3). Additionally, they were extremely contaminated by Cd in the core RXII at the depth

10–20 cm. Other sediment strata in cores RXII, RVII, and RIV were moderately to heavily contaminated by Zn (classes II–V) and heavily contaminated by Cd and Pb (classes IV–V). Sediments were usually uncontaminated or weakly contaminated by Cu (classes 0–II).

3.3. Diatom Analysis in Water and Sediments in Subsidence Ponds

Planktonic diatoms were represented by 51 taxa belonging to 26 genera. *Gomphonema* (9), *Nitzschia* (9), *Fragilaria* (5) were the most diverse genera. The most abundant taxa in ponds existing during the mine operation (DOWN) were: *Achnanthidium minutissimum* (CH3), *Staurosira binodis* (CH2), *Lemnicola hungarica* (CH2), *Nitzschia supralitorea* (CH1, CH3), and *Sellaphora nigri* (CH2). In the pond that emerged after the mining cessation (UP) the most common diatoms were *Achnanthidium minutissimum* (CH4), *Gomphonema parvulum* (CH5), *Hippodonta capitata* (CH4), and *Planothidium frequentissimum* (CH4). They reached at least more than 10% of relative abundance in at least one sample.

Diatoms in the sediment were much more diverse in number of taxa. Totally, 230 taxa (66 genera) were found. The most abundant genera belonged to: *Nitzschia* (29), *Gomphonema* (16), and *Fragilaria* (14), but they were much more diverse in number of species than plankton. Among the most abundant taxa in the DOWN pond have been found: *Achnanthidium minutissimum* (RVI, RVII), *Meridion circulare* var. *circulare* (RVI, RVII), *Sellaphora nigri* (RVI), *Staurosira venter* (RXII), *Surirella brebisonii* var. *kuetzingii* (RVII), whereas in the pond that emerged after mine closure (UP) *Gomphonema utae* (RIV), *Planothidium lanceolatum* (RIV), *Staurosira venter* (RI) occurred in the largest amounts. *Staurosira venter* in core RXII showed over 90% dominance.

3.4. Cladocera Analysis in Water and Sediments in Subsidence Ponds

Planktonic Cladocera were represented by 13 taxa belonging to five families (Bosminidae, Chydoridae, Daphniidae, Eurycercidae, Polyphemidae). Daphniidae (5 taxa: *Ceriodaphnia quadrangula*, *Daphnia pulex*, *Moina micrura*, *Scapholeberis mucronata*, *Simocephalus vetulus*) and Chydoridae (5 taxa: *Coronatella rectangula*, *Alonella exigua*, *A. nana*, *Chydorus sphaericus*, *Pleuroxus truncatus*) were the most diverse, whereas the other families were represented by only one taxon. The highest density of total Cladocera was noted in CH1 pond while *Daphnia pulex* had the highest density during the summer in CH3 (121 ind./dm^3), CH5 (117 ind./dm^3), CH1 (267 ind./dm^3) ponds. In the UP pond, 8 taxa were found, against 12 taxa in the DOWN ponds. The number of cladoceran taxa in each plankton sampling point ranged from 4 to 9 (Table 3).

Table 3. Number of taxa and density of Cladocera in water and sediment of subsidence ponds.

Title	Plankton			Sediment		
	Sampling Point	Number of Taxa	Density of Total Cladocera in Water (ind./dm^3; min.–max.)	Core	Number of Taxa	Range (min.–max.) of Total Cladocera (ind. 1 cm^3) in Sediments
UP	CH4	7	2–99	RIV	7	4–20
	CH5	4	1–132	RI	5	1–6
DOWN	CH1	8	2–278	RVI	3	3–8
	CH2	9	7–47	RXII	13	24–111
	CH3	6	2–131	RVII	2	1–6

Cladocera remains in the sediments of the subsidence ponds were represented by 15 taxa belonging to three families (Bosminidae, Chydoridae, Daphniidae). Chydoridae was the most diverse family (11 taxa), other families had fewer taxa (Bosminidae-1; Daphniidae-3). The number of cladoceran taxa per sediment core ranged from 2 to 13 (Figure 3, Table 3). The highest density of total Cladocera in subsidence ponds was noted in the core RXII (111 ind./cm^3) (DOWN). *Alona* sp. (33 ind./cm^3) and *Chydorus sphaericus* (21 ind./cm^3) had the highest density in this core. In subsidence ponds (in plankton and sediment) we found 8 taxa in the UP pond characterized by through-flow of river water and 14 taxa in DOWN ponds characterized by stagnant water conditions and littoral zone overgrown with macrophytes. In all ponds we found taxa belonging to five genera: *Acroperus*, *Alona*, *Bosmina*, *Coronatella*, *Chydorus*, and *Daphnia*.

The pond (CH2; RXII; Table 3) had the highest diversity of Cladocera taxa in comparison with the other DOWN reservoirs. Total density of Cladocera remains in sediments (ind./cm^3) varied from 1 in UP pond (RI) to over 100 individuals in core XII in DOWN ponds (Table 3). In the UP pond Cladocera assemblage was rather poor and its density was not higher than 20 ind./cm^3. *Chydorus sphaericus* was dominant, present in all studied ponds. *Chydorus sphaericus* is known in pelagic and littoral zones, and its high density is characteristic for eutrophic and polluted water. The highest density of this species was observed in the core RXII (21 ind./cm^3), in the pond of mining area (DOWN) whereas, a much smaller density, below 10 ind./cm^3 was observed in cores of the pond formed after the mining period (UP) (Figure 3).

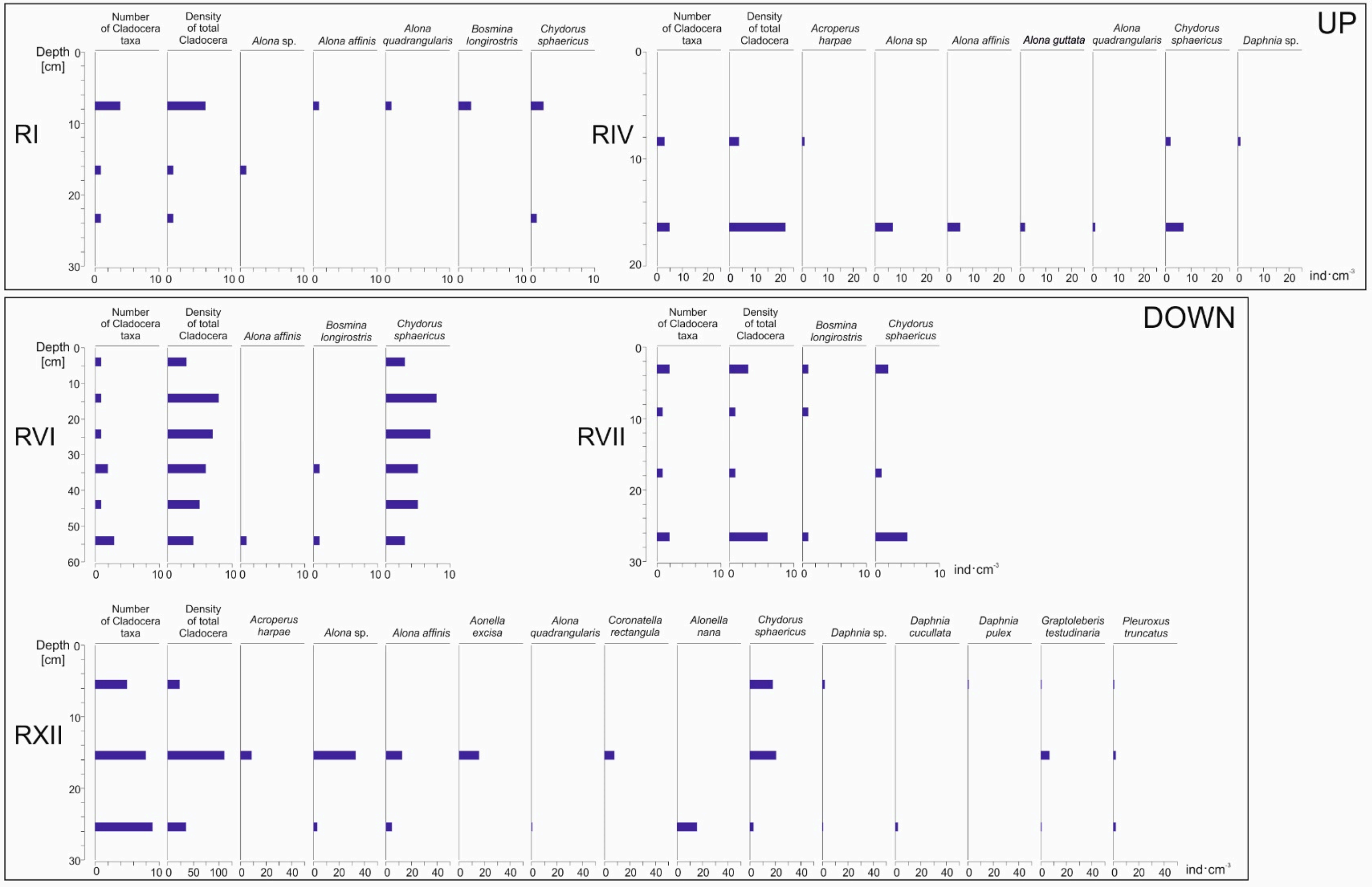

Figure 3. Density of Cladocera taxa in sediment cores (ind./cm^3) from subsidence pond created after mine was closed (UP) and ponds existed during mine exploitation (DOWN).

3.5. Relationship between Diatoms, Cladocera, and Environmental Variables in Subsidence Ponds

The highest Shannon (H′) diversity rates (up to 4) occurred for plankton and for diatoms of six sediment samples from UP and DOWN ponds. Values of the index for Cladocera community were different from those obtained for diatoms. Moreover, the high H′ diversity rates of Cladocera communities were observed in the two sediment cores (up to <1) from water bodies which were formed after mining (UP), as well as in a one plankton sample and in a one sediment core from the DOWN pond (Table 4).

Table 4. Shannon (H′) diversity index value in investigated plankton samples and sediment cores. (UP-subsidence pond formed after mine was closed; DOWN-subsidence ponds formed during the peak of Zn and Pb ore exploitation).

Title	UP				DOWN			
	Plankton	Index Value	Core	Index Value	Plankton	Index Value	Core	Index Value
Diatoms	CH4	2.945	RIV	3.671	CH1	3.105	RVI	3.426
	CH5	3.304	RI	3.296	CH2	2.483	RXII	2.423
					CH3	2.746	RVII	3.698
Cladocera	CH4	0.782	RIV	1.611	CH1	0.740	RVI	0.356
	CH5	0.558	RI	1.520	CH2	1.382	RXII	2.125
					CH3	0.498	RVII	0.591

The analysis of similarities of the plankton community (diatoms and cladocerans) ordered communities without distinguishing groups with respect to their similarity. This was caused by the different dominance of the identified species structure of diatoms and cladocerans at the sampling sites. The three closely situated DOWN ponds were characterized by stagnant water and were formed during the mine exploitation (D-CH1, D-CH3, D-CH2). The furthest two sampling sites were situated in a pond formed after the mine was closed, and the pond was characterized by a flow-through of water (U-CH4, U-CH5) (Figure 4).

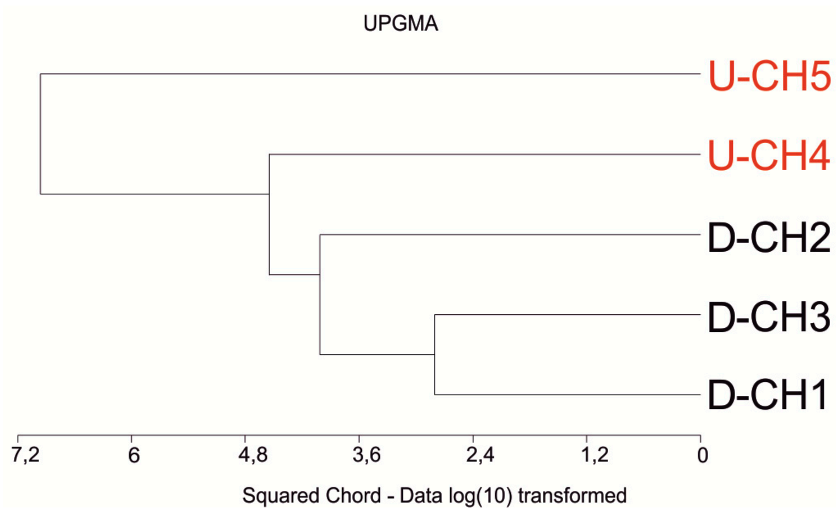

Figure 4. Dendrogram of similarities constructed for diatom and Cladocera in plankton samples from pond, created after mine was closed (UP) (U-CH5, U-CH4) and ponds existed during mine exploitation (DOWN) (D-CH3, D-CH2, D-CH1).

There were statistically significant correlations between the abundance of particular species of diatoms and cladocerans and physico-chemical data (also heavy metals) of water as well as the abundance of diatoms and cladocerans and heavy metal concentrations in the sediment cores of UP and DOWN ponds (Tables 5–8).

In the UP pond we did not find a significant correlation between heavy metals in water and planktonic diatoms. However, the highest and significant Spearman's rank correlation were found for *Aulacoseira ambigua*, *Pseudostaurosira brevistriata,* and *Staurosirella pinnata* (all for Zn dissolved), *Melosira varians* (negative correlation), *Navicula cryptocephala* (both for Pb dissolved) and significant negative correlation for *Cyclotella meneghiniana* (Cd dissolved) (Table 5A). In the sediment from the UP pond, significant positive correlations (Spearman's rank order) were found between *Aulacoseira ambigua* and Cu and between *Encyonema ventricosum* and Zn and Cd. Other species of diatoms negatively correlated with the heavy metal content in sediment (Table 6A).

In the DOWN ponds (existing during the mine operation) the highest Spearman's rank order correlation (significant) were found for ten planktonic diatom species and metal content in water, among which the highest were for: *Achnanthidium minutissimum* (Pb), *Cocconeis placentula* var. *placentula* (Pb), *Fragilaria* cf. *famelica* (Cd), and *Staurosira venter* (Cd, Zn). Other diatoms were negatively correlated with heavy metal content (*Melosira varians*, *Nitzchia archibaldii*, *N. linearis*, *N. palea* var. *debilis*, *Sellaphora saugerresii*, *Thalasiosira pseudonana*) (Table 7A).

In the sediment samples of DOWN ponds, such diatoms as *Achnanthidium minutissimum* (Cd, Cu), *Asterionella formosa* (Zn), *Aulacoseira granulata* (Cd), *Encyonopsis cesatii* (Pb), *Fragilaria* cf. *gracilis* (Cd, Zn, Pb), *Meridion circulare* var. *circulare* (Zn, Cu, Pb), *Navicula veneta* (Cu), *Nitzchia archibaldii* (Cd, Zn, Cu, Pb), *N. capitellata* (Zn, Cu, Pb), *N. gracilis* (Cd, Zn, Pb), *N. palea* var. *debilis* (Zn), *N. subacicularis* (Pb), *Planothidium lanceolatum* (Cd, Zn, Pb), *Sellaphora nigri* (Cd, Zn, Pb, Cu), *Surirella angusta* (Pb), *S. brebisonii* var. *kuetzingii* (Cd, Zn, Pb, Cu), and *Ulnaria ulna* (Zn, Pb) significantly correlated with the heavy metals. Among 25 significantly correlated species *Nitzchia archibaldii*, *Sellaphora nigri*, and *Surirella brebisonii* var. *kuetzingii* have highly significant positive correlation values with all analyzed heavy metals (Table 8A). The highest correlation values with Pb concentrations were obtained for *Encyonopsis cesatii* (0.793), *Sellaphora nigri*, and *Surirella brebisonii* var. *kuetzingii* (0.756 and 0.839 respectively). The remaining diatoms were negatively correlated with the heavy metals content (Table 8A).

Table 5. Spearman's rank order correlation for planktonic diatoms (A) and cladocerans (B) from pond created after mine was closed (UP).

(A) Diatoms

Species	Conductivity	pH	Cd Dissolved	Pb Dissolved	Zn Dissolved	*Aulacoseira ambigua*	*Pseudostaurosira brevistriata*	*Nitzschia palea* var. *palea*
Aulacoseira ambigua	0.814				0.731			
Cyclotella meneghiniana			−0.812					
Melosira varians	0.774			−0.736		0.778		
Navicula cryptocephala				0.733				
Nitzschia capitellata		0.766						
Nitzschia palea var. *palea*	0.764						0.719	
Nitzschia supralitorea		−0.764						
Pseudostaurosira brevistriata					0.736	0.768		
Staurosira construens	0.764					0.768	0.839	0.839
Staurosirella pinnata					0.896			

(B) Cladocerans

Species	SO_4^{2-}	NO_3^{-}	PO_4^{3-}	*Craticula buderi*	*Craticula cuspidata*	*Fragilaria bidens*	*Gomphonema parvulum*	*Halamphora veneta*	*Navicula cryptocephala*	*Sellaphora nigri*	*Sellaphora saugerresii*
Bosmina longirostris						0.756					
Ceriodaphnia quadrangula									0.756		
Chydorus sphaericus				0.814				0.750		0.768	0.765
Daphnia pulex		0.826	0.802								
Moina micrura	0.764	0.764			0.751		0.733				
Pleuroxus truncatus									0.756		
Scapholeberis mucronata									0.750		

Table 6. Spearman's rank order correlation for diatoms (A) and cladocerans (B) from sediment core of subsidence pond (UP).

Species	Zn	Cd	Pb	Cu	*Caloneis lancettula*	*Diploneis fontanella*	*Encyonema perpusillum*	*Gomphonema utae*	*Staurosirella leptostauron*	*Surirella brebisonii* var. *kuetzingii*	*Tabellaria flocullosa*
(A) Diatoms											
Aulacoseira ambigua				0.845							
Caloneis lancettula	−0.828	−0.828		−0.828							
Diploneis fontanella	−0.820	−0.820			0.880						
Encyonema perpusillum	−0.828	−0.828		−0.828		0.880					
Encyonema ventricosum	0.820	0.820				−0.871					
Gomphonema utae				−0.812	0.840		0.840				
Navicula radiosa	−0.845	−0.845		−0.845	0.980	0.898	0.980	0.857			
Nitzschia acidoclinata	−0.845	−0.845		−0.845	0.980	0.898	0.980	0.857			
Pinnularia borealis	−0.845	−0.845		−0.845	0.980	0.898	0.980	0.857			
Punctastriata sp.	−0.845	−0.845		−0.845	0.980	0.898	0.980	0.857			
Staurosirella leptostauron			−0.845								
(B) Cladocerans											
Alona affinis			−0.845								0.836
Alona quadrangularis			−0.828						0.980		0.853
Chydorus sphaericus	−0.971	−0.971	−0.971	−0.883						−0.836	

Table 7. Spearman's rank order correlation for diatoms (A), cladocerans (B), and physicochemical parameters of water of subsidence ponds formed during the peak of ore exploitation (DOWN).

(A) Diatoms

Species	Cd Dissolved	Pb Dissolved	Zn Dissolved	*Achnanthidium minutissimum*	*Nitzschia palea* var. *debilis*	*Sellaphora saugerresii*
Achnanthidium minutissimum		0.587				
Cocconeis placentula var. *placentula*		0.643		0.866		
Fragilaria cf. *famelica*	0.601					
Melosira varians		−0.641				
Nitzchia archibaldii			−0.629			
Nitzschia linearis		−0.579				
Nitzschia palea var. *debilis*			−0.750			
Sellaphora saugerresii			−0.600			
Staurosira venter	0.600		0.729		−0.632	
Thalasiosira pseudonana			−0.646			0.774

(B) Cladocerans

Species	Hardness	NO_3^-	PO_4^{3-}	Cd Dissolved	*Craticula buderi*	*Fragilaria bidens*	*Navicula cryptocephala*	*Nitzchia archibaldii*	*Nitzschia linearis*	*Planothidium lanceolatum*	*Sellaphora nigri*	*Staurosirella pinnata*	*Surirella brebisonii* var. *kuetzingii*
Coronatella rectangula													
Alonella exigua		−0.640				0.634	−0.586						
Bosmina longirostris								0.591					
Ceriodaphnia quadrangula		−0.724	0.716		−0.623								
Chydorus sphaericus										−0.630		−0.619	−0.672
Daphnia pulex				0.637									
Pleuroxus truncatus	−0.795						−0.714				0.734		
Simocephalus vetulus				0.733					−0.603584				−0.641

Table 8. Spearman's rank order correlation for diatoms (A) and cladocerans (B) from sediment core of subsidence pond formed during the peak of ore exploitation (DOWN). Taxa name: diatoms—Ach.min: *Achnanthidium minutissimum*; Ast.for: *Asterionella formosa*; Aul.gra: *Aulacoseira granulata*; Coc.neo: *Cocconeis neodiminuta*; Enc.ven: *Encynema ventricosum*; Enc.ces: *Encyonopsis cesatii*; Eun.min: *Eunotia minor*; Fra.gra: *Fragilaria* cf. *gracilis*; Gom.bre: *Gomphonema brebissonii*; Gom.par: *G. parvulum*; Gom.sag: *G. sagitta*; Mer.cir.var.cir: *Meridion circulare* var. *circurale*; Nav.ven: *N. veneta*; Nit.arc: *N. archibaldii*; Nit.cap: *N. capitellata*; Nit.gra: *N. gracilis*; Nit.pal.var.deb: *N. palea* var. *debilis*; Nit.sub: *N. subacicularis*; Pla.lan: *Planothidium lanceolatum*; Sel.nig: *Sellaphora nigri*; Sta.ven: *Staurosira venter*; Sur.ang: *Surirella angusta*; Sur.bre.var.kue: *Surirella brebisonii* var. *kuetzingii*; Tab.fas: *Tabularia fasciculata*; Uln.uln: *U. ulna.* Cladocerans—Alo.sp: *Alona* sp.; Alo.aff: *A. affinis*; Alo.qua: *Alona quadrangularis*; Alo.exc: *Alonella excisa*; Alo.nan: *A. nana*; Bos.lon: *Bosmina longirostris*; Cor.rec: *Coronatella rectangula*; Dap.sp: *Daphnia* sp.; Dap.cuc: *D. cucullata*; Gra.tes: *Graptoleberis testudinaria*; Ple.tru: *Pleroxus truncatus*.

A. Diatoms																										
Species	**Zn**	**Cd**	**Pb**	**Cu**	**Ach.min**	**Ast.for**	**Aul.gra**	**Coc.neo**	**Enc.ces**	**Eun.min**	**Fra.gra**	**Gom.bre**	**Gom.par**	**Gom.sag**	**Mer.cir. var.cir**	**Nav.ven**	**Nit.arc**	**Nit.cap**	**Nit.gra**	**Nit.pal. var.deb**	**Nit.sub**	**Pla.lan**	**Sel.nig**	**Sta.ven**	**Sur.ang**	**Sur.bre. var.kue**
Ach.min		0.54		0.54																						
Ast.for	0.64																									
Aul.gra		0.59					0.53																			
Coc.neo	−0.52																									
Enc.ven		−0.57																								
Enc.ces	0.54		0.79																							
Eun.min			−0.59																							
Fra.gra	0.68	0.61	0.60		0.58																					
Gom.bre	−0.52	−0.55	−0.59	−0.55	−0.58		0.62			0.82	−0.59															
Gom.par			0.58							−0.54																
Gom.sag			−0.59							1.00		0.81	−0.55													
Mer.cir.var.cir	0.65		0.69	0.69						−0.59		−0.55	0.62	−0.59												
Nav.ven				0.58		0.61	0.53				0.55															
Nit.arc	0.57	0.62	0.52	0.58	0.83						0.78		0.57													
Nit.cap	0.67		0.56	0.52				−0.56					0.65		0.73											
Nit.gra	0.62	0.52	0.61								0.72															
Nit.pal.var.deb	0.61				0.52			−0.59					0.73		0.62		0.68	0.71								
Nit.sub			0.59							0.63									0.66							
Pla.lan	0.56	0.54	0.65			0.58			0.66	−0.55	0.62		0.71	−0.55			0.65			0.55						
Sel.nig	0.64	0.70	0.76	0.58	0.58			−0.59	0.66	−0.59		0.58	0.80						0.63	0.52		0.87				
Sta.ven	−0.60		−0.59		−0.65	−0.63			−0.54	0.59	−0.60		−0.57	0.59		−0.55	−0.78		−0.52	−0.64		−0.76	−0.76			
Sur.ang			0.55		0.66				0.64			−0.52	0.51				0.52			0.52			0.62	−0.57		
Sur.bre.var.kue	0.71	0.70	0.84	0.65					0.64	−0.59			0.61	−0.59	0.76			0.58				0.68	0.69		0.71	
Tab.fas				−0.54																	0.56					
Uln.uln	0.61		0.52															0.71		0.52					0.61	0.66

Table 8. *Cont.*

B. Cladocerans															
Species	**Zn**	**Cd**	**Pb**	**Cu**	**Ach. min**	**Eun.min**	**Fra. gra**	**Gom. bre.**	**Gom. sag**	**Mer.cir.var.cir**	**Pla.lan**	**Sel.nig**	**Sta.ven**	**Sur.ang**	**Sur.bre.var.kue**
Alo.sp			−0.59			0.99		0.80	1.00	−0.59	−0.55	−0.59	0.59		−0.59
Alo.aff			−0.56			0.84		0.65	0.84		−0.58	−0.57	0.60		−0.62
Alo.exc						0.63			0.68						
Alo.qua						0.73		0.62	0.68						
Cor.rec						0.63			0.68						
Alo.nan						0.73		0.62	0.68						
Bos.lon										0.61					
Dap.sp								0.80							
Dap.cuc						0.73		0.62	0.68						
Gla.tes		−0.52	−0.58	−0.53	−0.58	0.80	−0.59	0.98	0.81	−0.55		−0.59		−0.52	
Ple.tru	−0.52	−0.54	−0.60	−0.55	−0.58	0.84	−0.59	0.99	0.85	−0.57		−0.60		−0.53	−0.52

In the UP pond we found no correlation between heavy metals and cladocerans community in the water. In the water two species *Moina micrura* and *Daphnia pulex* were positively correlated with other physico-chemical variables. In the sediments of the UP pond a negative correlation was found only between heavy metals concentration and Cladocera species. *Chydorus sphaericus* negatively correlated with all heavy metals, but *Alona affinis* and *A. quadrangularis* negatively correlated with Pb. Moreover, different species of diatoms positively correlated with cladocerans species probably reflecting trophic relationship (Tables 5B and 6B).

In the most polluted DOWN ponds a positive correlation between the two species of Cladocera (*Daphnia pulex* and *Simocephalus vetulus*) with dissolved Cd in the water, as well as a negative correlation between four taxa (*Alona* sp., *A. affinis*, *Glaptoleberis testudinaria*, *Pleuroxus truncatus*) and Pb in sediments were found. Cladocera were also negatively correlated with Zn, Cd, and Cu in the sediments. The correlation between diatoms and cladocerans taxa were both negative and positive, which may indicate more complex trophic relationships in these ponds (Tables 7B and 8B).

Generally, DOWN and UP ponds were statistically different with respect to plankton density and physico-chemicals of the water (Table 2). The U Mann-Whitney test showed significant differences in the density of plankton between UP and DOWN ponds ($Z = 2.044452$, $p = 0.040$). The density was significantly higher in UP ponds. Moreover, U Mann-Whitney test showed statistical differences in the cadmium concentration in the sediments ($Z = 2.607971$, $p = 0.009$) between DOWN and UP ponds. Significant differences were found also between the number of diatom and cladoceran species ($Z = 3.152921$, $p = 0.001$).

Canonical correspondence analyses (CCA) revealed an influence of physicochemical variables and heavy metal concentrations in water and sediments on the distribution of diatoms and cladocerans communities. Statistically significant relationships were observed only in sediment cores (Figure 5).

The CCA model for diatoms and cladocerans in the pond sediments indicated statistically significant negative correlation with lead. The Monte Carlo permutation test showed statistical significance for both the first canonical axis ($F = 2.591$, $p = 0.008$) and for all canonical axes ($F = 2.174$, $p = 0.006$). In the CCA analysis, the first axis explains 32.7%, and the second axis 18.2% of the total variability of diatoms and claodocerans in the cores. The results of the stepwise forward selection of environmental variables showed that distribution of diatoms and cladocerans in the cores were related only to the content of Pb in the sediments and to the age of ponds. Figure 5 shows the group of organisms which were associated with a high content of Pb in sediments and the group of organisms which were associated with DOWN water bodies.

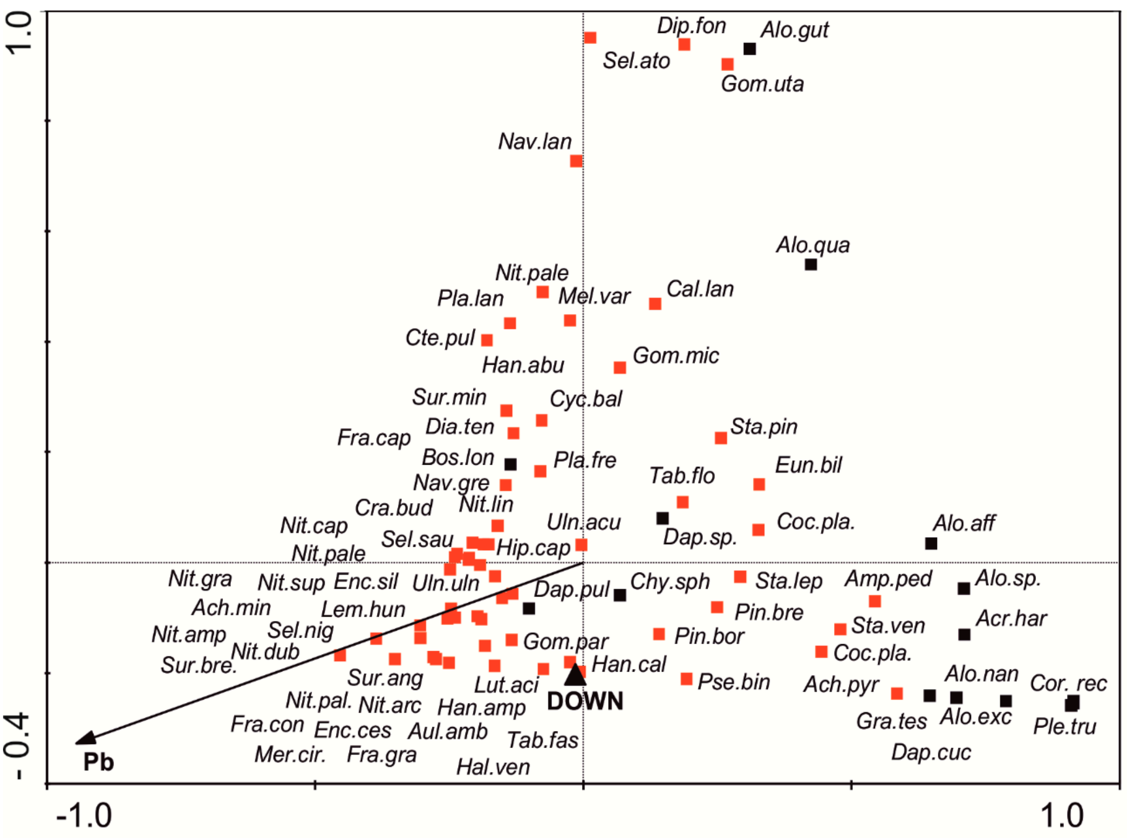

Figure 5. Canonical correspondence analysis (CCA) diagram for diatoms and cladocerans species. The biplot illustrates the relationship in sediments between the heavy metal concentration and diatoms and cladocerans. Taxa name: diatoms - Ach.min: *Achnanthidium minutissimum*; Ach.pyr: *A. pyrenaicum*; Amp.ped: *Amphora pediculus*; Aul.amb: *Aulacoseira ambigua*; Cal.lan: *Caloneis lancettula*; Coc.eug: *Cocconeis euglypta*; Coc.pla: *C. placentula* var. *placentula*; Cra.bud: *Craticula buderi*; Cte.pul: *Ctenophora pulchella*; Cyc.bal: *Lindavia balatonis*; Dia.ten: *Diatoma tenuis*; Dip.fon: *Diploneis fontanella*; Enc.sil: *Encynema silesiacum*; Enc.ces: *Encyonopsis cesatii*; Eun.bil: *Eunotia bilunaris*; Fra.cap: *Fragilaria capucina*; Fra.con: *Staurosira construens*; Fra.gra: *Fragilaria* cf. *gracilis*; Gom.mic: *Gomphonema micropus*; Gom.par: *G. parvulum*; Gom.uta: *G. utae*; Hal.ven: *Halamphora veneta*; Han.abu: *Hantzschia abudans*; Han.amp: *H. amphioxys*; Han.cal: *Hantzschia calcifuga*; Hip.cap: *Hippodonta capitata*; Mel.var: *Melosira varians*; Mer.cir: *Meridion circulare*; Lut.aci: *Luticola acidoclinata*; Lem.hun: *Lemnicola hungarica*; Nav.gre: *Navicula gregaria*; Nav.lan: *N. lanceolata*; Nit.amp: *Nitzchia amphibia*; Nit.arc: *N. archibaldii*; Nit.cap: *N. capitellata*; Nit.dub: *N. dubia*; Nit.gra: *N. gracilis*; Nit.lin: *N. linearis*; Nit.pac: *N. paleacea*; Nit.pad: *N. palea* var. *debilis*; Nit.pale: *N. palea* var. *palea*; Nit.sup: *N. supralitorea*; Pin.bor: *Pinnularia borealis*; Pin.bre: *P. brebissonii*; Pla.fre: *Planothidium frequentissimum*; Pla.lan: *P. lanceolatum*; Pse.bin: *Pseudostaurosira binodis*; Sel.ato: *Sellaphora atomoides*; Sel.nig: *S. nigri*; Sel.sau: *S. saugeressi*; Sta.ven: *Staurosira venter*; Sta.lep: *Staurosirella leptostauron*; Sur.ang: *Surirella angusta*; Sur.bre: *Surirella brebisonii* var. *kuetzingii*; Sur.min: *S. minuta*; Tab.flo: *Tabellaria flocullosa*; Tab.fas: *Tabularia fasciculata*; Uln.acu: *Ulnaria acus*; Uln.uln: *U. ulna*. Cladocerans - Acr.har: *Acroperus harpae*; Alo.sp.: *Alona* sp.; Alo.aff: *A. affinis*; Alo.gut: *A. guttata*; Alo.qua: *A. quadrangularis*; Alo.exc: *Alonella excisa*; Alo.nan: *A. nana*; Bos.lon: *Bosmina longirostris*; Chy.sph: *Chydorus sphaericus*; Cor.rec: *Coronatella rectangula*; Dap.sp: *Daphnia* sp.; Dap.cuc: *D. cucullata*; Dap.pul: *D. pulex*; Gra.tes: *Graptoleberis testudinaria*; Ple.tru: *Pleroxus truncatus*). Diatom taxa are marked in red color; cladocerans taxa are marked in black color.

4. Discussion

All the waters of the subsidence ponds on the Chechło River floodplain have higher values of conductivity and contents of ions SO_4^{2-}, Cl^-, PO_4^{3-} than small unpolluted water bodies in southern Poland [37]. However, these characteristics were similar to those in water bodies in the vicinity of another abandoned lead and zinc mine in Upper Silesia, in southern Poland [14]. Higher mean contents of above parameters in the UP pond (sites CH5 or CH4) were associated with the direct inflow of the Chechło River, contaminated by municipal sewages from the towns Trzebinia (~20,000 inhabitants) and Chrzanów (~40,000 inhabitants) in the upper section of the catchment [38]. Fluctuations of major ions and nutrients at site CH5 near the inflow of the Chechło River to the UP pond was probably mainly controlled by the river discharge because such changes were much lower in the downstream part of that pond. The differences of the same parameters in the water between sites CH1–CH3 of the DOWN ponds, are related to variable exchange rate between particular ponds and the Chechło River. The lowest concentrations of the above ions were found at site CH3 situated upstream from the inflow channel, in the most distant part of the pond. Inversely they were the highest at site CH2 of pond situated in proximity to the channel connecting the pond with the river. However, it should be emphasized that even during small floods all ponds (CH1–CH3) are flooded with river water.

Similarly to macroions, the total Cd, Pb, Zn, and Cu concentrations in the studied waters were predominantly close to values from industrialized areas [39]; nevertheless, they were much lower than in aquatic systems polluted by active Zn and Pb mining [40,41]. The concentrations of dissolved Cd did not exceed permissible values for priority substances, while dissolved Cu and Zn were not higher than permissible country values for substances harmful to the aquatic environment [42]. Only the concentrations of dissolved Pb at sites CH1–CH3 and CH5 exceeded the average annual permissible values (AA-EQS, 1.2 $\mu g/dm^3$) for priority substances, however they were still below maximum permissible values (Mac-EQS, 14 μg/L, [42]). Sporadically higher total Cd, Pb, and Zn concentrations in the UP pond (site CH5), could be related to runoff from industrialized part of the catchment during higher rainfalls. Higher or maximum Cd (total and dissolved) and Pb (total and dissolved) concentrations noted in late summer (August) could be related to a degradation of organic matter in ponds. The largest maxima of total and dissolved Cd and Pb occurred in DOWN ponds (CH2 and CH3) with the most contaminated sediments. A similar phenomenon was observed also in a fishpond of the nearby catchment and could reflect a release of these metals from sediments [39].

In contrary to the water, sediments were extremely contaminated by Cd, Pb, and Zn (according to values of I_{geo}, [35]) reaching levels found in water bodies affected by active and closed Zn and Pb mines [14,40,41,43]. This confirms that sediments of waters in mining areas act as long-term sinks for heavy metals [44,45]. Lower sediment contamination of the UP pond compared to the DOWN ponds (with some exceptions in the core RXII) is associated with the cessation of a discharge after closure of the mine. Low Cd, Pb, and Zn concentrations in the bottom strata of cores IV and XII indicate the lack of fluvial sediment deposition during mining era, before ponding of subsidence basins.

We studied changes of the planktonic and sedimentary diatoms over a temporal and spatial gradient of metal pollution in ponds affected by the operation of the ore mine because diatoms and cladocerans are excellent indicators of environmental change [4,46,47]. Most diatoms found in the plankton are tychoplanktic. That can be related to the small size of the ponds, which have the area not exceeding 4.5 ha of surface and 2 m depth [48]. However, diatom assemblages in Zn, Pb, Cu, and Cd polluted waters were generally resistant to observed metal concentrations because of large similarity to populations from non-contaminated waters.

The sampled sites from UP and DOWN ponds were grouped on a dendrogram of similarities constructed for diatoms and Cladocera in plankton samples where the CH4 and CH5 (UP) are clearly different from CH1, CH2, and CH3 (DOWN) (Figure 4). In the UP pond (sites CH5 or CH4), the content of nutrients and total hardness (Table 1) is higher than in DOWN ponds because of the inflow of municipal sewages from nearby towns. The site CH1 was rich in *Achnanthidium minutissimum*, *Gomphonema parvulum*, *Lemnicola hungarica*, *Nitzschia amphibia,* and *N. supralitorea*. All of them belong to mesosaprobic and indifferent-mesotraphentic diatom group. Their abundance was highest at site CH2. Presence of some of these species, like *Lemnicola hungarica*, *Nitzschia amphibia,* and *N. supralitorea* indicate their adaptation to metal-contaminated waters. *Achnanthidium minutissimum* is well-known from metal contaminated waters [49], where this diatom clearly increases in population size [21,50].

Another diatom, *Gomphonema parvulum* is also present numerously under these conditions and similarly to *Lemnicola hungarica* and *Nitzschia amphibia*, it is considered a good indicator of strong water pollution. The example of over average dissolved Cd and Zn content is the site CH5 in the UP pond (Table 1) dominated by *Gomphonema parvulum* and *Planothidium frequentissimum* known as metal resistant [50]. Also, the site CH3 (DOWN) with the highest average dissolved Pb content (Table 1) was dominated by *Achnanthidium minutissimum*, *Cocconeis placentula* var. *placentula*, *Gomphonema parvulum.* Moreover, the above mentioned diatoms that adopted well to metal pollution belong to *Cocconeis placentula* var. *placentula.* Important diatoms in UP pond included also *Melosira varians* (Table 5), a metal-resistant diatom [50].

Generally, our results suggest that diatoms common in the ponds are resistant to moderate metal contamination in neutral and alkaline waters (Table 1) and even at sites CH5, CH2, or CH4 (Tables 1 and 2) with metals content raised over average. No shift toward domination of metal-resistant species was noted. This is supported also by other works stressing the presence of high content of hardness-causing cations (e.g., Ca^{2+} and Mg^{2+}) as the factor mitigating the toxicity of metals in mine water [5,6]. Also, the dominance of more sensitive species (e.g., *Gomphonema utae*, *Meridion circulare* var. *circulare*, *Planothidium lanceolatum* and *Staurosira venter*) indicates good adaptation to metal-contaminated waters.

The cores from UP pond (RI and RIV) were dominated by mesosaprobous and meso-eutraphentic diatoms *Gomphonema utae*, *Planothidium lanceolatum*, and *Staurosira venter.* The diatom/metal correlations are significant for several taxa (Table 6A, Table 7A, and Table 8A). But the CCA analyses exhibited the highest (significant) importance of Pb concentrations on the distribution of investigated biota. Other metals were correlated to Pb, however their impact was not significant. The diatoms most positively correlated to increase of Pb content were *Achnanthidium minutissimum*, *Nitzschia amphibia*, *Sellaphora nigri*, and *Surirella brebisonii* var. *kuetzingii.*

The increase in the number of these diatoms in our metal polluted sediments corresponds well with another finding. *Achnanthidium minutissimum* is generally considered as an indicator of metals pollution and is often reported as predominant in lotic waters exposed to heavy pollution by metals [50]. However, the status of this species as an indicator of this type of pollution has been discussed for a long time (diatoms attached to the substrate are more resistant but the ability of mobile diatoms makes them more susceptible to toxic substances) [20,51].

The presence of *Sellaphora nigri* (as *Eolimna minima* sensu auct. nonnull.), the most common benthic species in European freshwaters, is related to human-induced of eutrophication, heavy metal pollution, and nutrient-rich environments [20]. *Surirella brebisonii* var. *kuetzingii* and *Nitzschia amphibia* are also known to prefer metal-contaminated waters [50] and they are widely distributed diatoms in eutrophicated inland waters.

The taxa, in which relative abundance decreased with raised Pb content were e.g., *Gomphonema utae*, *Staurosirella pinnata*, *Eunotia bilunaris*, and *Alona* spp. (Figure 5). High number of *Achnanthidium minutissimum* (formerly called *Achnanthes*) associated with the decrease of *Staurosira venter*, *Staurosirella leptostauron*, and *S. pinnata* (formerly called *Fragilaria*) fits Hill et al.'s [52] opinion, that *Fragilaria* dominates at the less metal impacted sites when *Achnanthes* dominates at the more impacted sites. Moreover, the largest population of *Staurosira venter* (over 90%), was observed in the core RXII—the least metal-polluted site (Figure 2). Several diatoms species are known as metal tolerant and pioneer, substrate-adherent species. Interesting and probably related to the neutral and alkaline reaction of waters is the almost complete lack of teratological forms. Many authors [50,53] suggest their occurrence as indicator of strong metal pollution.

The Cladocera showed evident alteration after mine closure. Because of the short period of time after finishing of the exploitation and poorly identifiable post-mining sediment strata, this change could be identified from comparing the sediment and planktonic organisms. Generally, planktonic Cladocera is a more differentiated group (5 family and 13 taxa) than in sediment (3 families and 15 taxa). There was also a shift of dominant organisms from *Alona* sp. and *Chydorus sphaericus* in sediments to *Daphnia pulex* dominating the present-day planktonic taxa. Such a change was observed also in the Lake Orta (Italy) by Jeppensen et al. [54] where during the period of toxic discharge, the only dominant species were *Chydorus sphaericus*, scarce *Bosmina,* and rare *Alona* spp. whereas, the lake recovery was signified by a return of *Daphnia pulex*. The result achieved for the studied ponds is probably related to the fact that in the UP ponds the river water flows through the center of the pond, whereas the DOWN ponds are supplied with river water by side channels, and have stagnant water with abundant macrophytes. Leppänen [4,10] in studies on mining pollution on *Bosmina longirostris* and *Chydorus sphaericus* underlined that those organisms tolerate mine water-impacted conditions. Some of authors mentioned also that *Ch. sphaericus* is tolerant to water pollution in a wide range of abiotic conditions [55], but the long-term exposure of this species to Cu can reduce its rate of population growth [56,57]. We observed strong negative correlation between heavy metals (Zn, Cd, Pb, Cu) and *Ch. sphaericus* in subsidence pond formed after the mining cessation with sediments less contaminated by heavy metals.

In the present study the Shannon (H’) index showed that much more diverse Cladocera communities occurred in plankton—but also in sediment cores of the subsidence pond formed after the mining cessation (UP)—than in older ponds (DOWN). These differences confirm the cladogram of similarities (constructed both for Cladocera and diatoms).

The change in abundance of some Cladocera correlates with water chemistry for: SO_4^{2-} (*Moina micrura*), NO_3^- (*Moina micrura*, *Daphnia pulex*), PO_4^{3-} (*Daphnia pulex*-UP) (*Ceriodaphnia quadrangula*-DOWN). A negative impact was noted only in DOWN ponds with NO_3^- and *Alonella exigua* and *Ceriodaphnia quadrangula*. The highest density of *Daphnia pulex* in water of DOWN ponds appear to be weakly impacted by heavy metals reflecting their adaptation to long-lasting contamination.

Pb was the most important metal that negatively impacted Cladocera in UP and DOWN ponds. Pb was not tolerated by *Alona*, *Chydorus*, *Graptoleberis,* and *Pleuroxus*. García-García et al. [58] confirm that high Pb concentration in water had a negative impact on *Diaphanosoma*, *Moina,* and *Alona*, excluding periods of raised water turbidity mitigating lead toxicity to cladocerans. In all subsidence ponds *Alona* and *Chydorus* were the dominant, and most abundant in studied sediments. The dominance of less sensitive species confirmed adaptation of cladocerans communities to chronic metal contamination [25]. Trophic relationship between diatoms and cladocerans were observed in sediment cores from UP and DOWN ponds. This is related to the ability of cladocerans to colonize in almost every type of freshwater.

Our research confirmed that heavy metal concentration in water from subsidence ponds had no influence on diatom and cladoceran communities, and recovery of the diatom and cladocerans communities is influenced by high heavy metal concentrations in the sediments of ponds in the river valley downstream of the mine waters discharge.

These results may be a key to understanding drivers for recovery of water ecosystems after long-term disturbances of their functioning.

5. Conclusions

This work presents important information on assessing the mine-water pollution impact on an aquatic ecosystem. In particular, it highlights the usefulness of diatoms and cladocerans as warning indicators of environmental change, supporting the use of multiple sediment proxies in paleolimnological pollution research. They provide information about the timing, direction, and magnitude of impacts caused by pollution events.

The analysis of plankton and remains of diatoms and cladocerans allowed to reconstruct pre-mining condition in the subsidence ponds. It also showed the conditions of the ponds when the Zn-Pb mine was operating and after it had been closed. The occurrence of different ecological groups of diatoms and cladocerans (diversity in taxa and in density) in the subsidence ponds revealed the changes in water quality during mine operation and afterwards.

Neolimnological studies describe the present conditions of biotic communities but paleolimnological information reveals past limnological conditions as an archive of environmental history.

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